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Research Article

Expression of Cyclin D1 in Oral Squamous Cell Carcinoma

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Abstract

Background: Cyclin D1 expression regulates normal cell cycle. Its deregulation or overexpression may cause disruption in the normal cell cycle control and lead to cancer progression. In this study, we aimed to study the expression of cyclin D1 in oral squamous cell carcinoma (OSCC) and find its association with the different grades of oral tumors, if any.

Methods: This cross-sectional study included 40 formalin-fixed paraffin-embedded tissue blocks specimens of OSCC with variable grades. The expression of cyclin D1 was evaluated through immunohistochemical (IHC) staining.

Results: A total of 40 (9 female and 31 male) samples were included, with a male-to-female ratio of 3.4:1. The age ranged between 25 and 90 years with an average age of 65.5 years. Twenty-five (62.5%) samples were diagnosed as well-differentiated squamous cell carcinoma (WDSCC) and fifteen (37.5%) as poorly differentiated squamous cell carcinoma (PDSCC). No cases of moderately differentiated squamous carcinoma were included in the study. The expression of cyclin D1 was detected in the cases of WDSCC and a lesser expression was seen in the PDSCC with a *P*-value of 0.0003, OR 1581 and 95% CI (29.8239 to 83810.7113).

Conclusion: Cyclin D1 is expressed in OSCC and stronger expression was detected in WDSCC.

Keywords: Cyclin D1, oral squamous cell carcinoma (OSCC), Toombak, Sudanese oral cancer, immunohistochemistry

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1. Introduction

Cyclin-dependent kinases (CDKs) are a family of serine/threonine kinases controlling progression through the cell cycle [1]. The cyclin-CDKs direct a linear progression of actions that transfer cells from a resting state (G0) to cell division (M). If any abnormalities occur in any of these phases, CDKs start a signal that triggers a cell cycle arrest until the issue is resolved. There are 11 cyclins found in human cells with many having subfamily members (e.g., D-type cyclin D1, D2, and D3) [2]. If a mutation arises, it

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Page 558

affects the cyclin function and can lead to derailed cellular proliferation and thus cancer. Cyclin D1 overexpression is important for the development and progression of several cancers including breast, esophagus, bladder, and lung [3–10]. Different studies have reported increased cyclin D1 in oral squamous cell carcinoma (OSCC) [11–15]. However, the association of cyclin D1 with pathological grading was inconclusive. Therefore, this study aimed to evaluate the expression of cyclin D1 in OSCC in Sudanese patients and its potential association with cancer grade.

2. Materials and Methods

This cross-sectional study included a total of 40 formalin-fixed, paraffin-embedded tissue samples histopathologically diagnosed with different grades of squamous cell carcinoma of the oral cavity. The samples were recruited from the upper aerodigestive tract (UADT) biobank in collaboration with the pathology department, Khartoum University during the years 2014–2016.

Then, the immunohistochemical (IHC) staining was carried out using the indirect streptavidin-biotin method. In the beginning, sections from each tissue block were cut to a thickness of 3 μm by rotary microtome, mounted in positively charged slides then de-paraffinized in an oven for 30 min and treated in xylene. Rehydration was carried out in graded ethyl alcohol (100%, 90%, 70%, 50%, respectively) and transferred into distilled water for 2 min. Antigen retrieval was performed by using a water-bath with a citrate buffer (pH 6.8). Then, slides were incubated for 10 min in 0.3% hydrogen peroxide to block endogenous peroxidase activity. The slides were then treated with anti-cyclin D1 primary antibody for 20 min and washed in phosphate buffer saline (pH 7.4). After that, the slides were treated with biotinylated secondary antibody for 20 min and then incubated in streptavidin-horseradish peroxidase for 15 min, washed in phosphate buffer saline (pH 7.4), incubated in 3-3 diaminobenzidine tetrahydrochloride (DAB) substrate solution for 7 min, and then washed in a running tap water. The slides were counterstained in Mayer's hematoxylin stain for 1 min, dehydrated in graded alcohol, cleared with xylene, and finally mounted in DPX mounting media [16]. All quality control measures were adopted; positive and negative control slides were used during IHC staining.

Detection of more than five cells with brown nucleus per one field is considered a positive result. Data analysis was done using the SPSS v.16. The mean frequency and Chi-square test values were calculated and P-value < 0.05 was considered significant.

3. Results

The study included 9 female and 31 male samples with a male-to-female ratio of 3.4:1. The age ranged between 25 and 90 years with an average age of 65.5 years. Twenty five (62.5%) samples were diagnosed as well-differentiated squamous cell carcinoma (WDSCC) and 15 (37.5%) as poorly differentiated squamous cell carcinoma (PDSCC) (Table 1). There was no moderately differentiated squamous cell carcinoma case at the time of the study. The expression of cyclin D1 was strongly detected in the cases of WDSCC, while a lesser expression was seen in the cases of PDSCC. Cyclin D1 expression showed a *p*-value of 0.0003, OR 1581, and 95% CI (29.8239 to 83810.7113) (Table 2).

4. Discussion

Cyclin D1 expression occurs during the G1 phase of the cell cycle. Its fundamental role is to integrate extracellular growth factor signals with the cell cycle regulatory mechanism [17–19]. Deregulation which leads to overexpression of cyclin D1 may shorten the G1 phase, increase cell proliferation, and reduce the need to growth factors [20], which in turn might result in an accumulation of unrepaired DNA mutations resulting in loss of cell cycle control and thus tumor formation [21].

In the present study, the average age of the patients was 65.5 years, which is the common age in patients diagnosed with oral cancer. Oral cancer develops mainly between the sixth and the seventh decades of life and incidence in younger people (aged <40 years) is infrequent [22 –28]. In the Arab countries, including Sudan, oral cancer patients were mostly in their 50s and 60s, and the rate at a younger age was reported [29].

In this study, males were three times more affected with OSCC than females, an observation reported by many investigators [30–35], including Sudanese scientists who had attributed males' increased exposure to their frequent use of *Toombak* [36, 31, 37].

Furthermore, it is known that SCC constitutes 90% of the oral cancer [38, 39] and that WDSCC was more common than other types, thus our findings were expected.

The study showed overexpression of cyclin D1 in 62.5% of cases examined. This was comparable with previous studies [40, 41, 42] who reported an overexpression in oral SCC in 68%, 63%, and 70.7% of the cases, respectively.

Here, and based on the grade of OSCC, cyclin D1 expression was mainly detected in WDSCC and a lesser expression in PDSCC. This observation is different from what was reported by Mate *et al.* in 1996 [43] who found cyclin D1 expression significantly

increases with the increase in differentiation of OSCC, however, similar to Saawarn *et al.,* who showed lesser expression with the advanced grades [44]. The diverse technical and biological reasons play a role in cyclin D1 expression which is why the role of cyclin D1 in cancer initiation and progression appears to be complex [45]. This could explain the contradicting prognostic significance of cyclin D1 [46–49].

On the other hand, HPV infection remains to be the best prognostic factor for head and neck cancers. This is because patients diagnosed with HPV infection have better clinical outcomes than other patients who are not [50]. This might reflect the fact that the cyclin D1 is rarely amplified in HPV-infected tumors [51, 52]. Although we do not have information on HPV infection on the studied samples, a previous study on Sudanese patients diagnosed with upper aerodigestive tract cancers showed 26 (17.3%) out of 150 patients were positive for HPV [53].

In conclusion, cyclin D1 can be used in the diagnosis of OSCC and its relationship with tumor progression needs to be further investigated.

Acknowledgements

Ethical Consideration

This project was approved by the National Research Ethical Review Committee Health Research Council, Republic of Sudan National Ministry of Health in 2014. All samples are part of the UADT cancer biobank. Informed consent was signed by all study participants.

Competing Interests

The authors declare that they have no competing interests.

TABLE 1: The different grades of oral cancer SCC.

Oral cancer grade	Number of diagnosed patients	Percentage (%)
WDSCC	25	62.5
PDSCC	15	37.5
Total	**40**	**100**

TABLE 2: The association of cyclin D1 and OC histopathology grade.

Cyclin D1 expression	Number of patients	Histopathology grade	P-value	OR	95% CI
Cyclin D1 strong expression	25	WDSCC	0.0003	1581	29.8239 to 83810.7113
Cyclin D1 weak expression	15	PDSCC			

Availability of Data and Material

Data published in this project will be available upon request; however, patients' identity will be blocked in order to protect the study participants.

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